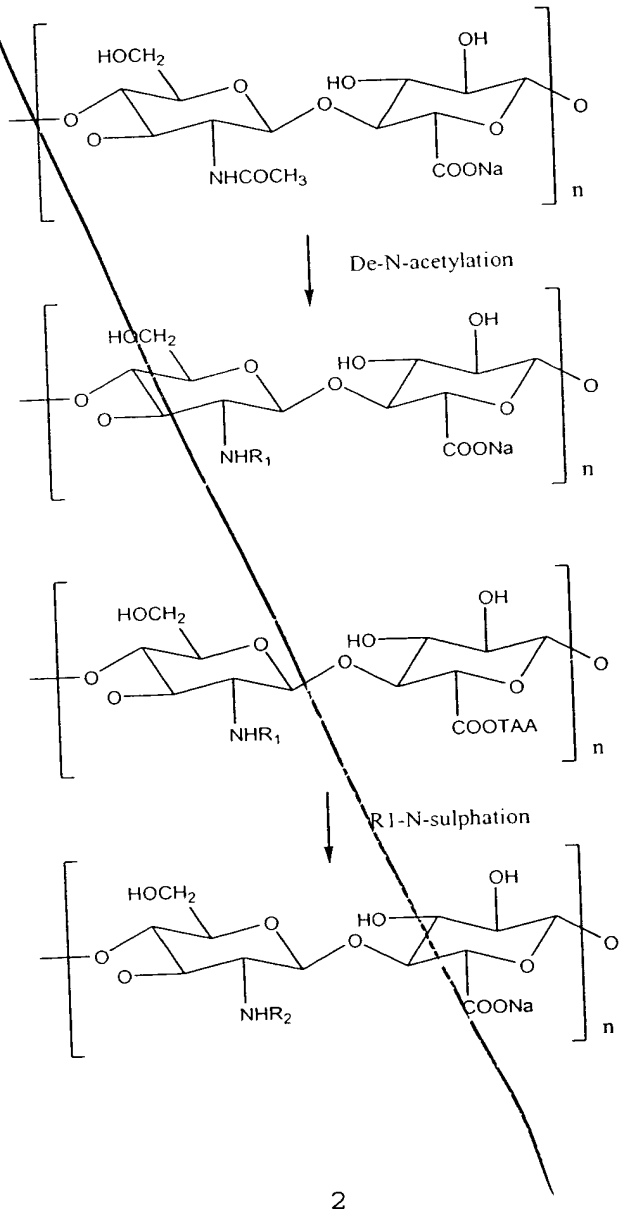
both types being disclosed in U.S.Patent No. 6,051,701, which is incorporated herewith by reference;

B_1) N-sulphated hyaluronic acids, and

B_2) N-sulphated hyaluronic acid derivatives,

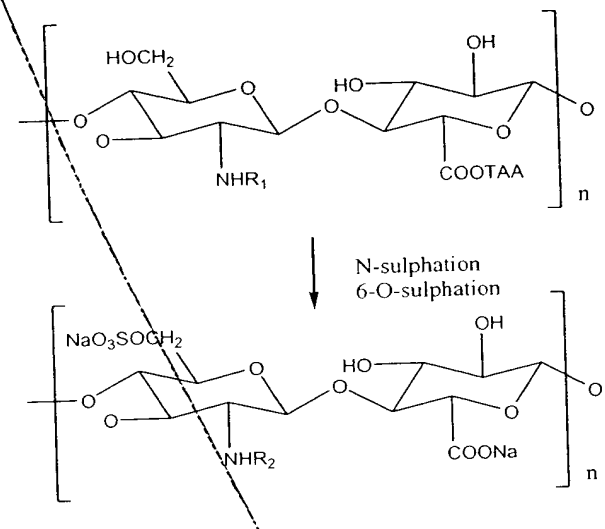
both types being obtainable by means of a controlled sulphation reaction on the amino group of glucosamine of hyaluronic acid, previously deacetylated according to the procedure described by P. Shaklee (1984) Biochem. J., 217, 187-197. The reaction proceeds as illustrated below:



n: from 12 to 12,500
R_1 = H, $COCH_3$
TAA = tetra-alkylammonium
R_2 = SO_3, $COCH_3$

Diagram 1

b) and c) mean the products of the chemical reaction illustrated in Diagram 1, wherein, besides the amino group of glucosamine, the primary hydroxy function of the same residue is also totally or partially involved in the sulphation reaction, as illustrated below:

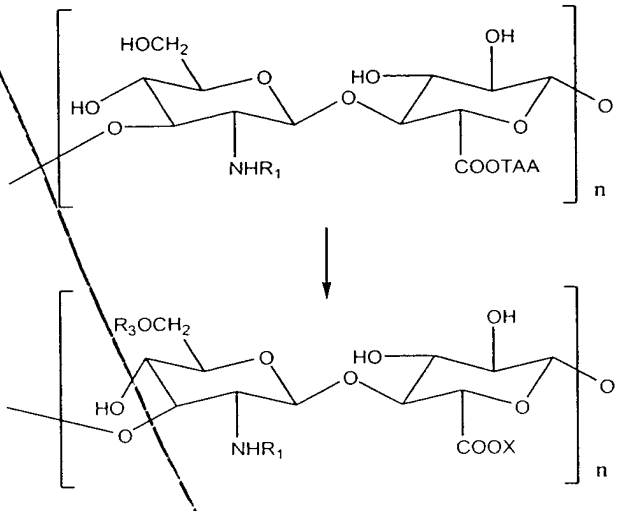


n: from 12 to 12,500
R_1 = H, $COCH_3$
TAA = tetra-alkylammonium
R_2 = SO_3, $COCH_3$

Diagram 2

The derivatives generated according to diagrams 1 and 2 can be used as intermediate reactants in the preparation of compounds, according to the procedure described in U.S. 4,851,521, wherein the carboxy function of the glucuronic residue of hyaluronic acid, partially 2-N-sulphated or partially 2-N-sulphated and

partially or totally 6-O-sulphated, is partially or completely reacted with alcohols of the aliphatic, aromatic, arylaliphatic, cycloaliphatic, heterocyclic series, producing the respective partial or total esters:



n: from 12 to 12,500

R_1 = H, $COCH_3$

TAA = tetra-alkylammonium

R_2 = SO_3, $COCH_3$

R_3 = SO_3, H

X = alcoholic residue, Sodium

Diagram 3

Moreover it is possible to use the synthetic derivatives according to diagrams 1 and 2 as intermediates in the preparation of crosslinked compounds, according to the procedures described in U.S. 5,676,964 and U.S. 4,957,744 respectively, wherein a part or all of the carboxy groups belonging to the D-glucosamine residue are reacted: i) using condensing agents with the alcoholic functions of the same polysaccharide chain or other chains, generating inner (or lactone) esters and intermolecular

esters; ii) with polyalcohols of the aliphatic, aromatic, arylaliphatic, cycloaliphatic, heterocyclic series, generating crosslinking by means of spacer chains.

B1

The above said sulphated compounds obtained according to the process of the present invention can be optionally salified with heavy metals, the heavy, metals being selected from the group of metal elements in the 4th, 5th and 6th periods of the periodic table, such as silver, iron, cobalt, copper, zinc, arsenic, strontium, zirconium, antimony, gold, cesium, tungsten, selenium, platinum, ruthenium, bismuth, tin, titanium and mercury.

Page 4, after line 26, insert:

The process for the preparation of the compounds B_1 and B_2 mainly consists of two steps, the first involving the controlled deacetylation of the natural polysaccharide, and the second involving the specific sulphation reaction of the primary hydroxyl or free amino functions of glucosamine.

B2

Fractions of hyaluronic acid from biological and fermentation sources, with a molecular weight of between 5,000 and 5,000,000 Da, preferably between 50,000 Da and 300,000 Da, are solubilized in hydrazine hydroxide with a purity of no less than 98%, in a concentration range of between 1 and 50mg/ml, preferably between 5 and 25 mg/ml. This solution is then supplemented with hydrazine sulphate in a weight/volume concentration varying between 0.1 and 3%, preferably 1%. The reaction is conducted within a temperature range of 40 to 90°C, preferably 60°C, under agitation, for as long as it takes to reach the desired degree of N-deacetylation.

Table 1 hereafter reports the yield expressed as the percentage of free amino groups, in terms of time expressed as hours of reaction:

Table 1

Test	Temperature(°C)	Time (hours)	N-deacetylation (%)*
DAc 1**	60°C	4	3

DAc 2	60°C	8	5
DAc 3	60°C	16	9
DAc 4	60°C	24	14
DAc 5	60°C	48	23
DAc 6	60°C	72	36

* The percentage of N-deacetylation is determined according to the method of J. Riesenfeld (Analy. Bioch. 1990, vol. 188, pages 383-389).

** DAc + N-deacetylation

The reaction is then stopped by precipitation with a polar solvent, preferably ethanol. The precipitate is partially vacuum-dried and treated with a solution of iodic acid with a molarity range of between 0.1 and 1M, preferably 0.5M, and lastly, with iodohydric acid at a concentration of 57% (w/v). The pH of the solution is maintained between 5 and 7 by adding a solution of sodium acetate (10% w/v).

The aqueous phase containing the modified polysaccharide is extracted by repeated treatments with diethylether and then, once the yellow color has completely disappeared, the solution is treated again with ethanol.

The precipitate which forms, after further drying at 40°C, is solubilized in water at a concentration of between 10ng/ml and 40 ng/ml, preferably 25 ng/ml, and the solution is percolated through a column containing an ion exchange resin activated with a tetra-alkylammonium hydroxide, where the alkyl residue of the quaternary ammonium is constituted by a chain of between 1 and 4 carbon atoms; tetrabutyl-ammonium hydroxide is preferably used.

The percolated product, represented by the quaternary ammonium salt of the modified polysaccharide, is then freeze-dried.

Preparation of: a) partially N-sulphated derivative (Method A)

The quaternary ammonium salt, preferably of tetrabutyl-ammonium,

of the partially deacetylated polysaccharide, is solubilized in a polar a solvent such as dimethyl sulphoxide, dimethyl formamide, dimethyl acetamide, N-methyl-pyrrolidone, preferably dimethyl formamide (DMFA), at a concentration of between 5 and 50mg/ml (preferably 25mg/ml).

The organic solution is supplemented with another solution obtained by a sulphating complex constituted by dimethylformamide sulphotrioxide (DMFA-SO_3), in DMFA, at a concentration varying between 50 and 200 mg/ml and preferably 100mg/ml. The quantity of complex to be used, expressed in moles of SO_3, proves surprisingly to be equivalent to the moles of amino groups released by the N-deacetylation reaction.

B2

The sulphation reaction proceeds at a temperature of between 0° and 20°C, preferably 4°C, for no longer than 45 hours and is then stopped by adding cold, distilled water.

The reaction solvent is first purified by precipitating the partially N-sulphated hyaluronic acid with ethanol and then dialyzing the resolubilized product with distilled water.

Lastly, the solution is freeze-dried and the solid product thus obtained undergoes chemical-analytical characterization to determine the degree of N-sulphation and the mean molecular weight (Table 2).

Table 2

Test	% deacetylation	% N-sulphation	mean MW (Da)
HA	0	0	165,000
HA-NS1	5.0(DAc2)	4.8	157,000
HA-NS2	14.2(DAc4)	13.9	147,000
HA-NS3	23.5(DAC5)	23.0	139,000
HA-NS4	36.1(DAc6)	34.2	124,000

HA = hyaluronic acid

HA-N-S = N-sulphated hyaluronic acid

Preparation of: b) partially 2-N-sulphated derivative (Method B)

The quaternary ammonium salt, preferably of tetrabutyl-ammonium, of the partially N-deacetylated polysaccharide is solubilized in a polar solvent such as dimethylsulphoxide, dimethylformamide, dimethylacetamide, N-methyl-pyrrolidone, preferably dimethylformamide (DMFA), at a concentration of between 54 and 50mg/ml, preferably 30 mg/ml.

The organic solution is supplemented with another solution obtained by solubilizing the sulphating complex constituted by dimethylformamide sulphotrioxide (DMFA-SO_3), in DMFA, at concentrations varying between 50 and 200 mg/ml and preferably 100 mg/ml. The quantity of complex used, expressed as moles of SO_3 prove surprisingly to be equivalent to the moles of amino groups released by the N-deacetylation reaction.

B2

The sulphation reaction proceeds at a temperature of between 0° and 20°C, preferably at 4°C for 4 hours. A solution prepared by solubilizing the pyridine-sulfotrioxide complex in dimethylsulphoxide in such a quantity that the ratio between the moles of SO_3 of the sulphating agent and the moles of $-CH_2OH$ is between 1.1 and 1.3. Larger quantities of reagent may favor any substitution reactions in other alcohol groups (secondary) of the polysaccharide chain.

The reaction the proceeds for another 16 hours at least after which it is stopped by adding cold distilled water.

All subsequent steps concerning the purification of the modified polysaccharide are those described in Method A.

The analytical characterization performed on the derivatives obtained confirmed that the sulphation method proves surprisingly not only to substitute all the amino groups obtained by the partial deacetylation, but also results in the complete substitution of the primary alcohol group of the glucosamine